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Chemical contamination alters the interactions between bacteria and phytoplankton --Manuscript Draft--

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Abstract:	<p>Bacteria and phytoplankton are key players in aquatic ecosystem functioning. Their interactions mediate carbon transfer through the trophic web. Chemical contamination can alter the function and diversity of phytoplankton and bacterioplankton, with important consequences for ecosystem functioning. The aim of the present study was to assess the impact of chemical contamination on the interactions between both biological compartments. Two contrasting marine coastal ecosystems, offshore waters and lagoon waters, were exposed to chemical contamination (artificial or produced from resuspension of contaminated sediment) in microcosms in four seasons characterized by distinct phytoplankton communities. Offshore waters were characterized by a complex phytoplankton–bacterioplankton network with a predominance of positive interactions between both compartments, especially with Haptophyta, Cryptophyta, and dinoflagellates. In contrast, for lagoon waters, the phytoplankton–bacterioplankton network was simpler with a prevalence of negative interactions with Ochrophyta, Cryptophyta, and flagellates. Contamination with an artificial mix of pesticides and trace metal elements resulted in a decrease in the number of interactions between phytoplankton and bacterioplankton, especially for offshore waters. Resuspension of contaminated sediment also altered the interactions between both compartments. The release of nutrients stored in the sediment allowed the growth of nutrient limited phytoplankton species with marked consequences for the interactions with bacterioplankton, with a predominance of positive interactions, whereas in lagoon waters, negative interactions were mostly observed. Overall, this study showed that chemical contamination and sediment resuspension resulted in significant effects on phytoplankton–bacterioplankton interactions that can alter the functioning of anthropogenic coastal ecosystems.</p>
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Chemical contamination alters the interactions between bacteria and phytoplankton

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Highlights

- Phytoplankton-bacterioplankton interactions were studied in lagoon and offshore waters
- The two ecosystems exhibited specific phytoplankton-bacterioplankton networks
- A more complex network was observed in offshore waters
- Positive interactions between phytoplankton and bacterioplankton dominated in offshore waters
- Contamination resulted in a simplification of the phytoplankton-bacterioplankton network

Outdoor microcosm incubation

1 **Abstract**

2 Bacteria and phytoplankton are key players in aquatic ecosystem functioning. Their
3 interactions mediate carbon transfer through the trophic web. Chemical contamination
4 can alter the function and diversity of phytoplankton and bacterioplankton, with
5 important consequences for ecosystem functioning. The aim of the present study was
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7 biological compartments. Two contrasting marine coastal ecosystems, offshore waters
8 and lagoon waters, were exposed to chemical contamination (artificial or produced
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19 between both compartments. The release of nutrients stored in the sediment allowed
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21 the interactions with bacterioplankton, with a predominance of positive interactions,
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25 functioning of anthropogenic coastal ecosystems.

26
27 **Keywords**

28 Pesticides, trace metal elements, coastal ecosystems, phytoplankton–bacterioplankton
29 networks

1 Introduction

In aquatic pelagic systems, phytoplankton and bacteria represent the most important producers of particulate organic material from inorganic and dissolved organic sources (Berglund et al., 2007). The organic matter can be transferred to higher trophic levels through different food webs, ranging from the microbial loop to the classic herbivore pathways depending on resource availability and prey size (Azam et al., 1983; Berglund et al., 2007). As a general rule in marine coastal and estuarine ecosystems, bacteria mediate more carbon than is produced *in situ*, resulting in net heterotrophy of the system (Morán et al., 2013). The ecosystem is thus depending on the availability of other sources of carbon such as recycled autochthonous carbon sources (Fouilland et al., 2014) or allochthonous terrestrial sources (Fouilland et al., 2018a; Morán et al., 2002). Since bacterioplankton and phytoplankton represent the first levels of pelagic marine food webs, any perturbation (natural or anthropogenic) impacting these key players is likely to impact the higher trophic levels and the ecosystem functioning depending on the strength and nature of the biological interactions within the aquatic trophic web (Sakka Hlaili et al., 2014).

Phytoplankton–bacterioplankton interactions have received increasing attention in recent decades. These interactions comprise four major modes as clearly described by Grossart and Simon (2007) and Amin et al. (2012): (1) a symbiotic association between phytoplankton bacteria and, in which bacteria benefit from phytoplankton exudates and phytoplankton growth is in turn favored by bacterial products (re-mineralized nutrients, vitamins, and other growth factors); (2) a parasitic association, where algae can inhibit bacterial growth by releasing antibiotic compounds, while bacteria can also act as parasites on phytoplankton leading to cell lysis and death of their hosts, (3) a commensal association with no positive or negative effect between both compartment; and (4) phytoplankton is only loosely associated with bacteria, thus allowing competition for limiting nutrients. The ecological relationships between phytoplankton and bacterioplankton represent the most important inter-organism

association in aquatic environments (Seymour et al., 2017). Phytoplankton–bacterioplankton interactions strongly influence carbon and nutrient cycles, regulate the productivity and stability of aquatic food webs (Seymour et al., 2017), and affect ocean–atmosphere fluxes of climatically relevant chemicals such as CO₂ (Cole, 1982) or dimethyl sulfide (DMS) (Yoch, 2002). The shared evolutionary history of these organisms (Cirri and Pohnert, 2019) has played an important role in shaping global biogeochemistry and aquatic ecosystem functioning (Seymour et al., 2017).

Anthropogenic perturbations like global warming (Hoppe et al., 2008), acidification (Hornick et al., 2017) or nutrient loading (Prieto et al., 2015) can alter the interactions between phytoplankton and bacterioplankton. For example, a nutrient increase can modify the metabolism of phytoplankton-associated bacteria and consequently the physiology of phytoplankton (Russo et al., 2016). Recent studies have shown that toxic contaminants such as herbicides can indirectly affect bacterial communities through their tight coupling with herbicide-sensitive phytoplankton (Goni-Urriza et al., 2018). Natural or artificial exposure to contaminants can also promote increased tolerance to toxic compounds within phytoplankton and bacterioplankton communities (Blanck, 2002). In addition, recent works have demonstrated that bacteria can increase toxic tolerance of their associated phytoplankton species in culture (Fouilland et al., 2018; Levy et al., 2009). Sensitivity of phytoplankton to contaminants is also influenced by the seasons, since seasonal environmental conditions can increase or decrease the success of tolerant species (Berard et al., 1999; Gregorio et al., 2012). Although the phytoplankton–bacteria relationships through metabolite exchanges are well studied, little is known regarding the impact of chemical contaminants on the interactions between phytoplankton and associated bacteria.

The aim of the present study was to assess the consequences of chemical contamination on the interactions between bacterioplankton and phytoplankton in two contrasting ecosystems characterized by distinct phytoplankton species according to season. For that purpose, microcosm incubations were performed during different seasons with offshore and lagoon waters exposed to a sediment elutriate or an artificial mixture of

contaminants mimicking the main contaminants present in sediment elutriate (pesticides and trace metal elements (TME)). Previous works have shown that chemical contamination and sediment resuspension impacted the coastal marine chemical environment differently (Bancon-Montigny et al., 2019) depending on the water masses (open or semi enclosed) with important consequences for ecosystem functioning (Pringault et al., 2020, 2016). The present study is based on the same sampling and experimental approach extensively described in Bancon-Montigny et al. (2019) and Pringault et al. (2020), dealing respectively with contaminant dynamics in water and plankton, and with biogeochemical coupling between bacteria and phytoplankton. Here we focus on the consequences of contamination for phytoplankton and bacterioplankton diversity, and aim to decipher the phytoplankton-bacterioplankton interactions and the resulting microbial networks.

2 Materials and Methods

2.1 Study sites and sampling

This study was performed in Southwestern Mediterranean ecosystems, the bay and the lagoon of Bizerte (Fig. S1, Supplementary materials), during four seasons, spring (April 2014), winter (February 2015), fall (November 2015), and summer (July 2016). Like most of the Mediterranean coastal lagoons, the Bizerte lagoon (lagoon station, L), to the North of Tunisia, is a polluted coastal ecosystem subject to intense industrialization, urbanization, and agriculture pressures, as well as pressures from commercial and naval shipping harbors (Pringault et al., 2020). Consequently, sediments are strongly contaminated by a wide range of pollutants, including metals and organic compounds (polycyclic aromatic hydrocarbons (PAHs) and pesticides) (Barhoumi et al., 2014a, 2014b; Triki et al., 2017). The Bizerte bay (offshore station, O) is less contaminated than the lagoon (Pringault et al., 2020), although polar pesticides including herbicides have been detected in the lagoon watershed (Mhadhbi et al., 2019) and local PAH contamination has been recorded in the effluent from an oil refinery located on the shore of the bay (Zrafi-Nouira et al., 2009, 2008). Water sampling was

carried out from 2 m depth in a lagoon station (station L, 37° 12' 43.96'' N, 9° 50' 79.78'' E) and an offshore station (station O, 37° 16' 46.46'' N, 9° 53' 50.98'' E) (Fig. S1, Supplementary materials) following the sampling procedure described in Pringault et al. (2016). Water samples were collected from 2 m depth, corresponding to the chlorophyll *a* maximum along the water column (Sakka Hlaili et al., 2007). Upon sampling, water was filtered through 200 µm mesh to remove larger particles and the metazooplankton thus minimizing grazing effects during incubation.

Sediment elutriate was obtained from polluted sediment resuspension following the protocol described by Bonnet et al. (2000) and recommended by the Environmental Protection Agency (US EPA). Briefly, polluted sediment was sampled using a Van Veen grab at 4 m depth in front of a cement factory in the lagoon channel (station CH, 37° 15' 40.22'' N, 9° 51' 30.49'' E Fig. S1), one of the most polluted sites in the Bizerte lagoon (Ben Said et al., 2010; Pringault et al., 2020; Triki et al., 2017). Overlying channel water was also sampled at the same time following the sampling procedure used for the offshore and lagoon waters. Just after sampling, the sediment was sieved (2 mm mesh) to remove large particles and stored in a cool box until its return to the laboratory. In the laboratory, sediment was mixed with channel water (1:4 w/v ratio), and sediment was gently stirred for 8 h. After a 12 h settling period, the overlying solution, thereafter called "elutriate", was smoothly siphoned out and stored in the dark at 4 °C until spiking in microcosms few days later (Pringault et al., 2016). The same protocol for sediment elutriate preparation was applied for the four studied seasons. This protocol has been successfully used to characterize the contamination level and toxicity of sediments using non-adapted species (Bonnet et al., 2000; Carr and Chapman, 1995).

2.2 Incubation procedure

During the four studied seasons, seawater was incubated for four days in 9 L glass microcosms (22.5 cm diameter and 23 cm height). The microcosms were covered with a quartz lid to allow full penetration of natural sunlight, including UV radiation,

according to the protocol described in Pringault et al (2020, 2016) and briefly detailed below. A series of triplicate microcosms was filled with 1.5 L of elutriate and completed with 4.5 L of sample water (L and O stations) to achieve a final dilution of 25% (contaminated water microcosms (CW). Another series of triplicate microcosms (artificial contaminated water microcosms (ACW)) was filled with 6 L (O and L stations) and spiked with an artificial solution of TME (Ni, Cu, Zn, Cd, As, Pb), and pesticides and some metabolites (acetochlore, alachlore, DCPU 1-(3,4-dichlorophenyl)urea, DIA deisopropylatrazine, diuron, linuron, and simazine). The final concentrations of metals and pesticides were adjusted to mimic the concentrations observed in elutriate (Table S1, Supplementary materials) (see Bancon-Montigny et al., 2019 for more details). This treatment was used to assess the impact of the main chemical compounds observed in the elutriate without the possible confounding effects of enhanced nutrients, DOC or benthic microorganisms that are released during sediment resuspension and present in the elutriate in the CW treatment. Lastly, a third series of triplicate microcosms (Control, C) was filled with 6 L of sample water (O and L stations). All microcosms were incubated during 96h outdoors under natural sunlight in a 3 m³ pool with circulating seawater (open system) to maintain *in situ* water temperature. Two light sensors (Skye, England), measuring incident ultraviolet A and B radiation (315–380 nm, SKU 420 and 280–315 nm, SKU 430, respectively) and photosynthetically active radiation (PAR; 400–700 nm, quantum SKP 215) were installed to monitor the light received at the surface of the microcosms.

2.3 Chemical analyses

Chemical analyses of dissolved organic carbon (DOC), Chlorophyll *a* (Chl*a*); the nutrients (SiOH₄, NH₄, NO₃, NO₂), and TME and organic contaminants (pesticides and PAHs) were performed using the analytical protocols described in Bancon-Montigny et al. (2019) and Pringault et al. (2016).

2.4 Phytoplankton diversity

Water samples (245 mL) immediately fixed with buffered formaldehyde at 2% final concentration were collected at the two stations O and L, and at the end of the 96h incubations in each microcosm. Species identification and counts were performed with a BX 60 Olympus microscope equipped with a Progress C3-cooled Janoptik camera following the procedure of Pringault et al (2020). Each species was determined to the lowest taxonomic level according to Tomas et al. (1997), Hoppenrath et al. (2009), Kraberg et al. (2010), Viličić (2014), and the WoRMS database (<http://www.marinespecies.org>). Counts were performed according to the Utermöhl's concentration technique (Utermöhl, 1958) under an inverted microscope (Olympus IX70). Each taxon was counted individually, except for Cyanophyceae spp, *Chaetoceros* spp, *Pseudo-nitzschia* spp, *Prymnesiophyceae* spp and *Euglenoides* spp which were grouped into global taxa due to the difficulties of species identification. Two groups of flagellates were distinguished by size class (< 10 µm and >10-15 µm).

2.5 Bacterioplankton diversity

Bacterioplankton was sampled at the two stations O and L, and at the end of the 96h incubations in each microcosm. Samples were processed as previously described (Goni-Urriza et al., 2018). Briefly, after water filtration (1000 ml) using sterile filters (0.2 µm porosity), filters were immediately frozen in liquid nitrogen and stored at -80 until DNA and RNA co-extraction. Nucleic acid extraction was performed coupling the Fast RNA ProSoil direct kit (MpBIO) with the AllprepDNA/RNA mini kit (QIAGEN). DNA contamination in RNA samples was eliminated with the RNase-Free DNase Set (QIAGEN). The RNA reverse transcription was performed using the M-MLV RT enzyme (Invitrogen) with 20-50 ng of RNA, RNase OUT (Invitrogen) and 50ng of random primers, according to the manufacturer's instructions.

The composition of the bacterial community was assessed targeting the V3-V4 region of the 16S rRNA gene as described elsewhere (Goni-Urriza et al., 2018). Briefly, PCR amplifications of extracted DNA and synthesized cDNA were performed with the

AmpliTaq Gold 360 Master mix (Applied Biosystems) and 343F-784R primers' set following the procedure described in Goni-Urriza et al. (2018). PCR amplicons were sequenced using the MiSeq 250-paired technology (Illumina). Raw sequences are available on the NCBI SRA database (National Center for Biotechnology Information Sequence Read Archive) under the accession number PRJNA391504.

QIIME pipeline was used to analyze raw sequences. Briefly, sequences with a quality score <20 were discarded before to remove chimeras (UCHIME, denovo). USEARCH61 was used to generate OTUs and UCLUST with Silva database (v. 119) to determine the affiliation of the OTUs' representative sequences. Prior to data normalization (rarefyseq.py), reads affiliated with chloroplasts and singletons were removed.

2.6 Statistical analysis

Phytoplankton and bacterioplankton species richness (S) was determined by the number of taxa (phytoplankton) and OTUs (bacterioplankton). The Shannon diversity (H) index was estimated using the following equation (Hill et al., 2003):

$$H = \sum_{i=1}^S P_i \log P_i$$

where P_i represents the relative abundance of species or OTU i in each sample and S the species richness. Analysis of variance (ANOVA) was performed to test the significance of the differences observed between treatments, stations and seasons. Prior to ANOVA, homogeneity of variance (Levene's test) and normality (Shapiro–Wilk test) were determined. When these conditions were not met, differences between treatments were tested using the non-parametric Kruskal–Wallis ANOVA test. A posteriori paired multiple-comparisons were then performed using Tukey's HSD test. All statistical tests were carried out with the level of significance set at $p < 0.05$. For multivariate analysis of the phytoplankton and bacterioplankton diversity, the triplicate data were averaged and the relative abundance was transformed with arcsin ($x^{0.5}$) to obtain a normal data distribution (Legendre and Legendre, 1998) according

to the procedure described in Pringault et al (2016). Three canonical correspondence analyses (CCA) were performed, following the recommendations Ramette (2007) using the MVSP v3.12d software (Kovach Computing Service, Anglesey Wales). The first two CCAs used either the 100 most abundant OTUs or the phytoplankton groups as response variables and concentrations of chemicals, TMEs, nutrients and DOC as explanatory variables. The explanatory variables with variance inflation factors (VIF) > 20 were removed to eliminate collinearity of environmental variables. For bacterioplankton diversity, only OTUs with at least ten reads were considered in order to reduce the effects of rare OTUs (Goni-Urriza et al., 2018). Since similar environmental variables were identified as structuring factors for bacterioplankton and phytoplankton communities, a third CCA was performed with the 100 most abundant bacterioplankton OTUs as response variables and the phytoplankton groups as explanatory variables, in order to determine whether phytoplankton were structuring the bacterioplankton communities. Spearman correlation was calculated with XLSTAT software (Addinsoft, Bordeaux France) between the phytoplankton groups and the 100 most abundant OTUs. Significant ($p < 0.05$) Spearman correlation values $> |0.8|$ were then used to design phytoplankton–bacterioplankton networks with the help of the CYTOSCAPE software. Phytoplankton species and bacterial OTU are represented as nodes, and node-node interactions (edges) represented as links (Shannon et al., 2003).

3 Results

3.1 *In situ* bacterioplankton and phytoplankton diversity

The two stations were characterized by distinct environmental conditions with clear seasonal effects for T °C and Chl*a*. Chl*a* concentrations did not exceed 1 $\mu\text{g L}^{-1}$ in offshore waters, with a minimum observed in winter and a maximum during spring. On the contrary, maximum Chl*a* was observed during fall in lagoon waters with a value close to 5 $\mu\text{g L}^{-1}$. Dissolved organic carbon was significantly ($p < 0.05$) higher in lagoon waters, with concentrations up to 2.5 mg C L^{-1} in winter, whereas in offshore

waters, DOC was around 1 mg C L⁻¹. Similarly, nutrient concentrations were higher in lagoon waters (Table S1, Supplementary materials). Pesticides were most abundant in Lagoon waters especially DIA or Simazine, as well as some TME, Ni, Zn or Mn (Table S1, Supplementary materials).

These distinct environmental conditions were concomitant to specific phytoplankton communities in offshore and lagoon waters with clear seasonal effects for both ecosystems (Fig. 1 upper panel). In spring and fall, dinoflagellates, with high abundances of *Gonyaulax* sp., *Heterocapsa* sp., and *Prorocentrum* sp. were the dominant phytoplankton group in lagoon waters representing up to 55% relative abundance (spring), while in offshore waters flagellates and dinoflagellates (mostly *Scrippsiella* sp.) accounted together for more than 70% of the relative abundance in both seasons. Summer was characterized by a strong dominance of Ochrophyta (93%) in the lagoon with a dense population of *Chaetoceros* sp. (68% of relative abundance), and to lesser extent in offshore waters (56%). During winter, Haptophyta (*Coccolithophorids* sp.) were the dominant (42%) phytoplankton taxa in offshore waters, while in the lagoon, flagellates (38%) and Ochrophyta (27%) accounted for up to 65% of the relative abundance. Diversity indices (Shannon index and species richness) exhibited distinct seasonal patterns with significant ($F_{3,20} = 58.25$, $p < 0.05$, $F_{3,20} = 3.42$ $p < 0.05$, for Shannon index and species richness, respectively) differences between lagoon and offshore waters (Fig. S2, Supplementary materials). Species richness (S) and Shannon index (H) were significantly higher ($F_{1,22} = 26.82$ $p < 0.05$, $F_{1,22} = 8.07$ $p < 0.05$, for Shannon index and species richness, respectively) in offshore waters with maximum values observed for fall ($H = 2.91 \pm 0.07$, $S = 27.7 \pm 5.5$), whereas the lowest values for the Shannon diversity index were observed during summer in both ecosystems.

A total of 203 562 distinct OTUs were identified from the 16S rDNA library in the whole dataset (*in situ* and microcosm incubation). As observed for phytoplankton, *in situ* bacterioplankton diversity also showed variations according to season and ecosystem (Fig. 1 lower panel). Gammaproteobacteria (dominated by *Alteromonas* sp., *Marinobacterium* sp., *Litoricola* sp., and SAR86 clade) represented the most dominant

groups irrespective of the season for both offshore and lagoon waters, with up to 42% of the relative abundance in offshore waters during winter. Flavobacteria (21–34%) and Alphaproteobacteria (12–26%) were the other dominant groups with a relative abundance greater than 10%. Regarding the less dominant groups, Cytophagia (1.6–4.9%) and Betaproteobacteria (2.2–3.8%) abundances were greater in lagoon waters, whereas Deltaproteobacteria (1.4–5.0%) and *Deferribacter* sp. (0.6–3.7%) abundances were higher in offshore waters. Diversity indices (Shannon index) exhibited distinct significant ($F_{3,20} = 6.51$, $p < 0.05$) seasonal patterns (Fig. S2) in lagoon and offshore waters, with minimum values during spring (4.30 ± 0.28 and 4.03 ± 0.05 for lagoon and offshore waters, respectively) and winter (4.20 ± 0.06 and 4.47 ± 0.04 for lagoon and offshore waters, respectively). Maximum values were observed during summer (6.75 ± 0.08 and 6.74 ± 0.12 for lagoon and offshore waters, respectively) and fall (6.74 ± 0.16 and 6.94 ± 0.12 for lagoon and offshore waters, respectively). No significant ($p > 0.05$) differences were observed for the Shannon index and species richness between lagoon and offshore waters.

3.2 Impact of contamination on bacterioplankton and phytoplankton communities

The impact of chemical contamination on the phytoplankton community was ecosystem and season dependent. As a general rule, the most pronounced effects on the relative abundance of the phytoplankton groups (compared to the control treatment) were observed with elutriate exposure especially during fall, whereas artificial contamination by a pesticide and TME mixture provoked minor changes in phytoplankton community structure (Fig. 2, upper panel). The changes in relative abundances were generally more marked in offshore waters relative to lagoon waters, especially in fall where flagellates (60% relative abundance) became the dominant group in the CW microcosm while they represented less than 10% of *in situ* relative abundance. When all seasons were taken into account, exposure to elutriate provoked a slight but significant ($F_{2,33} = 13.41$, $p < 0.05$) increase in species richness in lagoon waters, whereas no significant effect was observed for the Shannon diversity index. In contrast, in offshore waters, both treatments (ACW and CW) resulted in a significant

310 ($F_{2,33} = 16.58$, $p < 0.05$) reduction of the Shannon index (Fig. S3) and the specific richness
 311 ($F_{2,33} = 7.83$, $p < 0.05$) (Fig. S4). CCA performed with the phytoplankton groups as
 312 response variables, and the contaminants and nutrients as explanatory variables (Fig.
 313 S5), showed that the impact of contaminants on phytoplankton structure was less
 314 marked than that imposed by spatial and temporal effects. Seasons were discriminated
 315 along axis 1, whereas stations were separated along axis 2. Nitrate, ammonium,
 316 silicate, DOC, Ni, and Zn were identified as positive structuring factors for the
 317 phytoplankton community in the lagoon ecosystem, especially during winter and
 318 spring. In contrast, offshore phytoplankton communities were negatively impacted by
 319 Zn, Ni, pesticides, and Cu, especially during spring and summer (Fig. S5). Among the
 320 different phytoplankton groups, dinoflagellates and Haptophyta exhibited the lowest
 321 abundance in ACW and CW treatments relative to the control (Fig. S6), whereas
 322 Ochrophyta and Cryptophyta abundances were similar in ACW relative to the control
 323 value, with an even higher abundance in CW for Cryptophyta (Fig. S6).
 324 For the bacterioplankton community, the impacts of contamination followed the same
 325 patterns as those observed for phytoplankton, with a more marked effect for the
 326 sediment elutriate relative to the artificial mixture contamination (Fig. 2, lower panel).
 327 The relative abundance of Gammaproteobacteria significantly increased ($F_{2,33} = 21.40$,
 328 $p < 0.05$, $F_{2,33} = 56.63$, $p < 0.05$, for offshore waters and lagoon waters, respectively) in
 329 the CW treatment especially in fall and winter, irrespective of the ecosystem, while
 330 relative abundance of Alphaproteobacteria ($F_{2,33} = 14.07$, $p < 0.05$ for offshore waters)
 331 and Flavobacteria ($F_{2,33} = 23.24$, $p < 0.05$, $F_{2,33} = 78.28$, $p < 0.05$, for offshore waters and
 332 lagoon waters, respectively) significantly decreased. Nevertheless, no significant
 333 changes were observed for the diversity indices (Figures S3 and S4, Supplementary
 334 materials). As observed for the phytoplankton community, the consequences of
 335 contamination were more evident in the offshore station although changes were
 336 partially masked by the seasonal and spatial effects as indicated by the canonical
 337 correspondence performed with the 100 most abundant OTUs as response variables,
 338 and the contaminants and nutrients as explanatory variables (Fig. S5, Supplementary

materials). Interestingly, the structuring environmental chemical factors identified for the bacterioplankton community were roughly similar to those observed for the phytoplankton community. Lagoon communities were structured by nitrate, DOC, Ni, Zn, Cd, and Cu, especially during spring and winter, whereas they negatively impacted the offshore community in summer and fall.

3.3 Impact of contamination on bacterioplankton-phytoplankton interactions

Since similar environmental variables were identified as structuring factors for bacterioplankton and phytoplankton communities, CCA was performed with the 100 most abundant OTUs as response variables and the phytoplankton groups as explanatory variables (Fig. 3). The two first axes explained almost 30% of the variance observed. Seasons were separated along axis 1 and ecosystem along axis 2. The impact of phytoplankton groups on the bacterioplankton community was clearly season and ecosystem dependent. Winter bacterial communities were mostly explained by flagellates especially for offshore waters and, by Haptophyta and Euglenozoa for lagoon waters. In both ecosystems, summer communities were structured by Ochrophyta, whereas dinoflagellates were identified as a structuring factor during fall. In order to estimate the possible interactions between bacterioplankton and phytoplankton, Spearman correlation coefficients were calculated between the relative abundance of the 100 most abundant bacterioplankton OTUs and the phytoplankton groups observed at the end of the microcosm treatments for both ecosystems (Fig. 4). Spearman correlation coefficients were also calculated within the 100 most abundant bacterial OTUs, in order to assess interactions among the bacterioplankton (Fig. S7, Supplementary materials). Highly significant Spearman correlations (> 0.8) were then used to draw bacterioplankton–phytoplankton networks as a function of the microcosm treatment for lagoon (Fig. 5) and offshore waters (Fig. 6). The nature (positive or negative) and the number of significant interactions ($p < 0.05$) were strongly dependent on the ecosystem and the microcosm treatments (Fig. 4). In the control treatment, positive interactions dominated, especially in offshore waters (451 positive vs. 113 negative) and to a lesser extent in lagoon waters (384 positive vs. 227

negative). Differences were also observed depending on the phytoplankton groups: Cyanobacteria, Bigyra, and Haptophyta showed mostly positive interactions with bacterioplankton, whereas a predominance of negative interactions was observed for flagellates and Ochrophyta, especially in lagoon waters. Contamination effects on the bacterioplankton–phytoplankton networks depended on the impacted ecosystem. In offshore waters, contamination resulted in an increase of negative interactions concomitant with a reduction of positive interactions., irrespective of the microcosm treatment (Fig. 6 and Fig. 4 lower panel). Interestingly, negative interactions became dominant for dinoflagellates, Chlorophyta, and Haptophyta in the CW treatment, whereas these groups exhibited a predominance of positive interactions with bacteria in control conditions (Fig. 6). In contrast, for lagoon waters, no marked changes for positive and negative interactions were observed between control and CW treatments (Fig. 4), whereas exposure to artificial contamination resulted in a similar decrease for both positive and negative interactions (Fig. 5 and Fig. 4 upper panel). Among the Gammaproteobacteria, the dominant bacterial genus, *Marinobacterium* sp. exhibited positive interactions with several phytoplankton taxa, irrespective of the treatments, whereas *Litoricola* sp. appeared less associated with phytoplankton (Table S2, Supplementary materials). Interactions among the most abundant OTUs were largely dominated by positive interactions (Fig. S7, Supplementary materials) irrespective of the treatments and the sampling site. Interestingly, for both ecosystems, chemical contamination and sediment elutriate resulted in an increase of interactions between OTUs; the large proportion of positive interactions (79–85%) observed in control microcosms remained similar in ACW and CW treatments.

4 Discussion

The aim of the present study was to assess the consequences of chemical contamination on bacterioplankton-phytoplankton interactions in two contrasting ecosystems, a semi enclosed lagoon ecosystem versus an open coastal ecosystem. The four seasons were studied to address the eventual modifications of the microbial community

composition driven by seasons. It is noteworthy, however, that the four seasons were studied from April 2014 to July 2016, excluding so the possible seasonal succession and the "ecological memory" of the microbial community between two consecutive seasons (Padisák et al., 2010). The two Mediterranean ecosystems exhibited contrasting environmental conditions during the four seasons studied with marked differences in chemical contamination (Table S1, Supplementary materials); the lagoon waters were significantly more contaminated than the offshore waters (Bancon-Montigny et al., 2019; Pringault et al., 2020). According to the concept of pollution-induced community tolerance that can be triggered by the chemical environment and the possible selection of tolerant species (Blanck, 2002), these two contrasting environments allowed testing the hypothesis that the microbial response to chemical contamination would be different in the two sampling sites, with possible consequences for the interactions between the two key players of the marine trophic web.

4.1 *In situ* phytoplankton and bacterioplankton diversity

The number of *in situ* phytoplankton species (129) identified by microscopy was in the same order of magnitude as that observed in similar Mediterranean ecosystems (Draredja et al., 2019; Percopo et al., 2011) or in other coastal regions (Srichandan et al., 2019) using the same counting technique. Similarly, the number of OTUs (19 169 for the *in situ* database) estimated by the 16S rRNA gene considered as a proxy of the bacterioplankton richness, was comparable to bacterial specific richness estimates determined with similar molecular tools in other coastal ecosystems (Goni-Urriza et al., 2018; Mohapatra et al., 2020). As generally observed in Mediterranean coastal ecosystems (Pulina et al., 2018; Quero et al., 2017), phytoplankton and bacterioplankton diversity exhibited clear seasonal variations in lagoon and offshore waters. Surprisingly, higher phytoplankton diversity indices were observed during spring and fall, seasons where phytoplankton blooms are likely to occur in Mediterranean coastal ecosystems (Ribera d'Alcalà et al., 2004); algal blooms often being concomitant with low phytoplankton diversity (Lehtinen et al., 2017; Livingston, 2007). Phytoplankton diversity was significantly higher ($p < 0.05$) in offshore waters

relative to lagoon waters, contrasting that observed in other lagoons where phytoplankton diversity in the enclosed system can be higher (Srichandan et al., 2019) or equivalent (Minicante et al., 2019) to their adjacent waters. These contrasting results show the particular trophic status played by semi enclosed ecosystems relative to their counterpart adjacent marine waters (Newton et al., 2014), differences that might be explained by the anthropogenic pressure exerted on enclosed systems that can result in a reduction of diversity. Both ecosystems shared common bacterioplankton species (1 739 OTUs) and phytoplankton species (51 species) such as OTUs 17 or 19 affiliated to the SAR 11 clade, the dinoflagellate *Heterocapsa minima*, or the diatom *Cylindrotheca closterium*, suggesting connectivity between lagoon and offshore waters (Minicante et al., 2019). Nevertheless, the environmental conditions were sufficiently different to allow the occurrence of distinct phytoplankton communities (48 and 30 species uniquely observed in offshore and lagoon waters, respectively) and bacterioplankton communities (9 033 and 8 397 OTUs specifically observed in offshore and lagoon waters, respectively). In fact, despite a large open channel to the coastal sea, the watershed influence and the very long water residence time (> 200 days) observed in the Bizerte lagoon (Béjaoui et al., 2017) relative to the offshore adjacent waters (< 20 days) (Sayol et al., 2013) allows accumulation of nutrients, DOC, and chemical contaminants within the lagoon (Bancon-Montigny et al., 2019; Pringault et al., 2020). Moreover, salinity and temperature variations showed distinct patterns with higher salinity and temperature during spring and winter in offshore waters compared to the lagoon ecosystem, whereas the opposite trends was observed during summer where temperature and salinity values were maximal in the semi enclosed system (Béjaoui et al., 2016; Pringault et al., 2020). The dissimilarities observed for bacterioplankton and phytoplankton communities between lagoon and offshore waters might result in distinct responses to anthropogenic pressure such as chemical contamination.

4.2 Contamination impact on bacterioplankton and phytoplankton communities

The impact of chemical contamination on bacterioplankton and phytoplankton communities was more marked in offshore waters than in lagoon waters with a clear

seasonal effect. A reduction of phytoplankton diversity concomitant to changes of phytoplankton structure was observed during spring and fall, especially upon sediment elutriate exposure (CW treatment). As a general rule, the structural changes were less pronounced when the communities were exposed to the artificial mixture of pesticides and TME (ACW treatment) than when exposed to sediment elutriate. The artificial contamination was designed to mimic the concentrations of pesticides and TME observed in sediment elutriate (Bancon-Montigny et al., 2019), but did not include increased levels of other elements such as nutrients, DOC or benthic microorganisms that were present in the sediment elutriate. This artificial cocktail resulted in a significant reduction of metabolic activities involved in the carbon cycle (Pringault et al., 2020, 2016), especially in offshore waters. Nevertheless, the toxic effects observed on microbial metabolic activities were alleviated when the toxic chemical compounds were accompanied by high concentrations of nutrients and DOC, chemical conditions observed in sediment elutriate (Bancon-Montigny et al., 2019). This exogenous supply of nutrients may promote the growth of nutrient limited phytoplankton species, especially during summer and winter, the post bloom periods, resulting in an increase of diversity and structural changes of the community. Similarly, the exogenous DOC supply by sediment elutriate may promote the growth of heterotrophic bacteria (Uchimiya et al., 2016), loosening their dependency on phytoplankton exudates to sustain their carbon requirements.

Enclosed systems under anthropogenic pressure, such as lagoons, promote favorable conditions for selective pressure of resistant species to chemical contamination according to the pollution-induced community tolerance concept proposed by Blanck et al. (1988) and illustrated with benthic phototrophic biofilms (Belando et al., 2017) or meiofauna (Boufahja and Semprucci, 2015). Dense populations of the diatom *Chaetoceros* sp. were observed in the lagoon ecosystem especially during summer, representing up to 68% of the total relative abundance. The *Chaetoceros* genus is considered one of the most abundant diatoms in marine phytoplankton (De Luca et al., 2019) and this phytoplankton species has been proposed as a biological indicator

for detecting environmental stress, considering its ability to thrive in contaminated marine environments (Verlecar et al., 2006). In this vein, the Gammaproteobacteria *Marinobacterium* sp. or *Alteromonas* sp. were often observed *in situ* in the lagoon waters with a relative abundance often close to 1%, and their abundance could reach up 10% (*Alteromonas* sp.) in contaminated microcosms, either by sediment elutriate (CW) or by the artificial mixture of pesticides and TME (ACW). The great abundance of *Marinobacterium* sp. and *Alteromonas* sp. under chemical contamination is in agreement with previous studies reporting their dominance in bacterial communities exposed to various stressors, including chemical contamination by oil or hydrocarbons (Catania et al., 2015; Dos Santos et al., 2011; Yakimov et al., 2005) or eutrophication (Wang et al., 2020). In addition, the great abundance of *Alteromonas* sp. in the ACW treatment confirms that this species could be used as a bioindicator of metal polluted environments (Selvin et al., 2009). The difference in phytoplankton or bacterioplankton communities observed in offshore and lagoon waters with the possible selection of resistant species in the enclosed ecosystem might affect the interactions between bacterioplankton and phytoplankton, considering that the microalgae–bacteria association is species dependent (Seymour et al., 2017).

4.3 Contamination and bacterioplankton-phytoplankton interactions

Phytoplankton–bacterioplankton interactions and the resulting networks were assessed with the Spearman correlation calculated with abundance data, thus allowing detection of positive (mutualism or symbiose) or negative (resource competition, parasitism, or predation) interactions among taxa (Goni-Urriza et al., 2018), bearing in mind the limitations of using co-occurrence as a proxy for ecological interactions (Blanchet et al., 2020; Freilich et al., 2018). This similarity based technique is often employed to construct microbial networks in aquatic ecosystems (Mikhailov et al., 2019; Zhu et al., 2018) although more complex modeling approaches allow distinguishing unidirectional ecological interactions (such as amensalism) from bidirectional ecological interactions (such as competition and mutualism) or

commensalistic association with no negative or positive effects between the considered species (Faust and Raes, 2012).

The lagoon ecosystem and its adjacent open waters were characterized by distinct phytoplankton–bacterioplankton interactions. In offshore waters, positive interactions largely dominated over negative interactions (451 positive vs. 113 negative) resulting in a complex bacterio–phytoplankton network. For the lagoon ecosystem, the proportion of negative interactions was higher (227 negative vs. 384 positive) drawing a simpler phytoplankton–bacterioplankton network, involving less bacterial species relative to the offshore situation. A similar tendency was observed in the Venice lagoon, with less interactions within the microbial communities in benthic and pelagic lagoon biotopes relative to the adjacent open waters (Minicante et al., 2019; Quero et al., 2017), suggesting that environmental heterogeneity in these adjacent and connected ecosystems can be strong enough to allow for ecological segregation (Minicante et al., 2019).

In both ecosystems, negative interactions were mainly observed in contaminated treatments (CW and ACW) with dinoflagellates whereas positive interactions dominated in control treatments. Negative correlations may indicate competition, inhibition, or predation among taxa. Dinoflagellates are known to produce toxin compounds resulting in allelopathic interactions with bacteria through cell lysis, blistering, or growth inhibition (Legrand et al., 2003; Paul et al., 2007). Nevertheless, dinoflagellates can also be specifically associated through positive interactions with particular bacterial species, such as *Roseobacter* sp. or *Marinobacter* sp. (Jasti et al., 2005). In contrast, bacterial interactions with Cryptophyta were dominated by positive correlations, especially in offshore waters in control conditions, as also observed in freshwater ecosystems where this phytoplankton group can form dense blooms (Mikhailov et al., 2019) knowing that this mixotrophic species can also be considered as bacterivores (Grujic et al., 2018). Interestingly, this mixotrophic regime might be controlled by the presence of contaminants, since the negative interactions between Cryptophyta and prokaryotes in ACW and CW treatments suggest an increase of

predation, as previously demonstrated by Losey & Denno (1999). Chemical contamination as well as sediment elutriate provoked significant changes in bacteria–phytoplankton interactions resulting in a simplification of the microbial pelagic network. A similar simplification effect of oil contamination was also observed in soils with a reduction of interactions and connectivity among bacteria (Liang et al., 2016). Anthropogenic stressors are known to impact the functioning of the aquatic primary producers and consequently, primary production is more sensitive to chemical contamination than respiration (Johnston et al., 2015). Reduction of phytoplankton biomass upon chemical contamination has been observed with a wide range of contaminants including metal (Rochelle-Newall et al., 2008), pesticides (Pelletier et al., 2006), plastic derivatives (M’Rabet et al., 2019), PAHs (Ben Othman et al., 2018; Hjorth et al., 2008, 2007), or with a contaminant mixture (Pringault et al., 2020). These inhibitory effects of chemical contaminants on the primary producers alter the synchrony between the phytoplankton and bacterioplankton (Goni-Urriza et al., 2018). Indeed, a reduction of phytoplankton biomass upon chemical stress, as observed in the ACW treatment, will also reduce the interactions with bacterioplankton, considering the interdependency observed between both compartments, especially in open waters where phytoplankton and bacterioplankton can be strongly coupled (Morán et al., 2002; Pringault et al., 2020).

In contrast to the artificial chemical contamination with TME and pesticides, exposure to sediment elutriate provoked a strong stimulation of phytoplankton biomass (Pringault et al., 2020) concomitant with a shift in the phytoplankton community structure, especially in offshore waters. The important release of dissolved organic matter (DOM) and nutrients from sediment resuspension (Bancon-Montigny et al., 2019) alleviated the toxic effects of chemical contaminants present in the sediment elutriate. Interestingly, the stimulation of phytoplankton biomass (Pringault et al., 2020) was concomitant with a reduction of phytoplankton diversity, leading to a simplification of the microbial network in lagoon and offshore waters. Phytoplankton blooms are known to strongly impact the interactions with bacterioplankton (Teeling

et al., 2012), especially in the case of harmful algal bloom (Paerl and Otten, 2013). A reduction of phytoplankton diversity during blooms has been already observed (Livingston, 2007), with no negligible impacts for the diversity of the associated bacterial community (Wemheuer et al., 2014), and for the interactions among phytoplankton species depending on which taxa dominates the bloom (Escalas et al., 2019). With phytoplankton and bacteria associations being species dependent (Grossart et al., 2005; Seymour et al., 2017), a shift (natural or anthropogenic triggered) in the phytoplankton community structure can impact the bacterial association, as differences in the phytoplankton community structure can lead to changes in quality and quantity of exuded organic matter that can be mineralized by bacteria for their growth (Kent et al., 2007, 2004; Liu et al., 2014). In addition, sediment resuspension provoked strong DOM release but also a significant increase in bacterial biomass, both conditions resulting in stimulation of the heterotrophic metabolism (Pringault et al., 2020). The simplified networks observed in CW treatments relative to the control clearly showed that this external supply of exogenous bacteria and dissolved organic matter decreases the interdependency between phytoplankton and bacterioplankton. Bacteria (indigenous and exogenous) have access to an external carbon source, so loosening their dependency on phytoplankton exudates to sustain their carbon demand, while interactions among bacteria increased. Increasing bacterial cooperation and association is important to allow biodegradation of recalcitrant biopolymers (Ebrahimi et al., 2019) such as those found in sediment-derived DOM, a complex mixture of organic compounds with different lability properties (Wu et al., 2018). Such a “priming effect” mechanism was widely reported in soils but rarely in marine waters (Bianchi, 2011; Blanchet et al., 2017).

5 Conclusion

The present study clearly showed that a semi-enclosed ecosystem and its adjacent open waters exhibited distinct phytoplankton and bacterioplankton communities that differ depending on season. These differences result in specific networks, suggesting

that environmental heterogeneity can be strong enough to allow for ecological segregation despite sea-lagoon connectivity. Offshore waters were characterized by a complex phytoplankton–bacterioplankton network dominated by positive interactions between primary producers and bacteria, while the semi-enclosed ecosystem harbored a simplified network where negative interactions were more prevalent. Chemical contamination resulted in a decrease of phytoplankton biomass especially during the bloom periods (spring and autumn) in offshore waters, leading to a simplification of the phytoplankton–bacterioplankton network dominated by negative interactions. Nutrients and DOM released by sediment resuspension alleviated the toxic effects of chemical compounds (mainly pesticides and TME) present in the elutriate, provoking stimulation of phytoplankton and bacterial biomass concomitant with a significant shift in the phytoplankton community structure and reduction of phytoplankton diversity. These structural changes favored the loosening of the bacteria–phytoplankton coupling (Pringault et al., 2020) with strong consequences for the trophic web and ecosystem functioning.

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Figure legends

Figure 1: Relative abundances of groups of phytoplankton and bacterioplankton observed *in situ* in the two sampling stations during the four seasons of the study.

Figure 2: Relative abundances of groups of phytoplankton and bacterioplankton observed at the end of the microcosm incubation as a function of treatments during the four seasons. C: Control, ACW: Artificial Contaminated Water and CW: Contaminated Water with sediment elutriate.

Figure 3: Correspondence analysis with the 100 most abundant OTUs and the phytoplankton groups as environmental variables measured at the end of the microcosm incubations. C: Control, ACW: Artificial Contaminated Water and CW: Contaminated Water with sediment elutriate.

Figure 4: Significant interactions ($p < 0.05$) between phytoplankton groups and the 100 most abundant OTUs estimated from Spearman correlations (Positive or Negative) as a function of the microcosm treatments. C: Control, ACW: Artificial Contaminated Water and CW: Contaminated Water with sediment elutriate.

Figure 5: Simplified network between phytoplankton groups and bacterial OTUs (indicated by numbers) in Lagoon station as a function of microcosm treatments. Green lines indicate significant ($p < 0.05$) positive correlations (Spearman) and red lines indicate significant ($p < 0.05$) negative correlations. Solid lines indicate bacterial interactions with other OTUs and with phytoplankton groups, dotted lines indicate interactions between phytoplankton groups. Only correlations $> |0.8|$ are indicated. C: Control, ACW: Artificial Contaminated Water and CW: Contaminated Water with sediment elutriate. See materials and methods for more details.

Figure 6: Simplified network between phytoplankton groups and bacterial OTUs (indicated by numbers) in Offshore station as a function of microcosm treatments. Green lines indicate significant ($p < 0.05$) positive correlations (Spearman) and red lines indicate significant ($p < 0.05$) negative correlations. Solid lines indicate bacterial interactions with other OTUs and with phytoplankton groups, dotted lines indicate interactions between phytoplankton groups. Only

1014 correlations $> |0.8|$ are indicated. C: Control, ACW: Artificial Contaminated Water and CW:
1015 Contaminated Water with sediment elutriate. See materials and methods for more details.
1016

CRedit author statement

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Figure 1

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Figure 2

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Figure 3

LAGOON

CONTROL

ACW

CW

OFFSHORE

CONTROL

ACW

CW

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